学術論文

Effects of Dietary Purple Rice on Plasma and Liver Lipid Levels in Rats[*1]
ラットの血漿および肝臓脂質レベルに対する食餌紫黒米の作用

Masashi KAWASAKI,[*2,3] Yuko SATO,[*2] Megumi CHIDA,[*2] and Chisato HATAKEYAMA[*2]
川崎雅志，佐藤裕子，千田めぐみ，畠山千怜

The effects of the ingestion of a dietary purple rice, Asamurasaki, on plasma and liver lipid levels were studied in rats. Rats were fed purple rice based on the amount of rice humans ingest in proportion to their total food intake. The plasma total cholesterol concentration in the groups fed both high content purple rice and low content purple rice were significantly higher than that in the control group. At the same time, both the high content purple rice and low content purple rice feeding significantly enhanced the plasma high-density lipoprotein (HDL)-cholesterol concentration. The plasma thiobarbituric acid-reactive substance (TBARS) value in the group fed the low content purple rice was not significantly different from the control group, although the high content purple rice feeding significantly decreased the plasma TBARS value. These results suggest that the purple rice may exert an antiatherosclerotic action along with the increase in the plasma HDL-cholesterol concentration, and an antioxidative action through the suppression of the plasma TBARS value.

Keywords: *purple rice, plasma lipid, liver lipid*
紫黒米，血漿脂質，肝臓脂質

INTRODUCTION

Grains are a very important food and energy source throughout the world. Some grains have become a subject of interest because of their dietary functions. We previously reported that buckwheat hot-water extract significantly decreased the plasma very-low-density lipoprotein plus low-density lipoprotein (VLDL+LDL)-cholesterol concentration in a basal diet, and significantly suppressed the liver triglyceride content and weakened the fatty liver in a cholesterol-loaded diet.[1)] Dietary proso millet protein concentrate significantly increased the plasma high-density lipoprotein (HDL)-cholesterol concentration in mice.[2,3)]

Rice has been a staple food in Japan from ancient times, and the consumption of rice is very high. Some types of rice are pigmented, and in Japan, red rice and purple rice are cultivated. The red rice contains a reddish brown pigment in the pericarp and in the seed coat of its unpolished rice. The pigment of the red rice includes catechin and catecholtannin, which are polyphenolic compounds. The pigment of the purple rice is anthocyanin. Purple rice is utilized as a natural food colorant, and in the production of red boiled rice and colored glutinous rice cakes.

The present study examined the effects of the ingestion of dietary purple rice, Asamurasaki, on the plasma and liver lipid levels in rats. One group of rats was fed the same number of calories of purple rice as human feeding of rice in all food intakes. The purple rice is usually cooked with white rice, and the purple rice is 10% of the total rice cooked. Therefore, the other group of rats was fed purple rice at a caloric level which was 10% of that consumed by humans eating rice.

MATERIALS AND METHODS

Animals and diets. This animal experiment was carried out in accordance with the standards relating to the care and management, *etc.* of experimental animals (Notification No. 6, March 27, 1980 of the Prime Minister's Office, Japan).

Male Wistar rats (3 wks old, Charles River Japan Inc., Kanagawa, Japan) were individually housed in stainless steel cages with wire bottoms in an air-conditioned room at a temperature of 22 ± 2℃, a relative humidity of 60 ± 5%, and a 12-h light cycle (0800-2000). They were fed a stock pellet diet (MF; Oriental Yeast Co., Tokyo, Japan) followed by a basal diet for 3 d. Subsequently, the rats were divided into three groups with similar body weights and were fed the basal (Control group (n = 6)) or experimental diets containing the purple rice (Asamurasaki), which was ground. The

*1 This study was conducted as a part of the Special Training of Food and Nutrition Major, Science of Living Department.

*2 Food and Nutrition Major, Science of Living Department.

*3 Corresponding author.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; TBARS, thiobarbituric acid-reactive substance; VLDL, very-low-density lipoprotein.

Table 1. Composition of experimental diets (g / 416 kcal).

Ingredients	Control	Low content purple rice	High content purple rice
Casein [1]	20	19.7	17.1
α-Cornstarch [1]	13.2	13.2	13.2
Cornstarch [1]	39.75	36.3	5.7
Sucrose [2]	10	10	10
Cellulose powder [1]	5	4.8	2.7
Soybean oil [1]	7	6.8	5.2
Mineral mixture (AIN93G composition) [1]	3.5	3.5	3.5
Vitamin mixture (AIN93 composition) [1]	1	1	1
Choline bitartrate [3]	0.25	0.25	0.25
L-Cystine [3]	0.3	0.3	0.3
Purple rice [4]	-	4.9	48.6

[1] Oriental Yeast Co., Tokyo, Japan.
[2] Nissin Sugar Manufacturing Co., Tokyo, Japan.
[3] Wako Pure Chemical Industries, Osaka, Japan.
[4] Tada Natural Farm, Iwate, Japan.

compositions of the basal and experimental diets are shown in Table 1.[4)] In the present study, the rats were fed the purple rice on the basis of human ingestion of rice energy. In humans, about 41.6% of food energy is ingested from rice in daily life. The energy of the basal diet is 416 kcal/100 g in the present study, so the caloric intake from rice is equal to 173 kcal/100 g. Therefore, the experimental diet of one group was supplemented with 48.6 g purple rice (High content purple rice group (n = 5)). (The energy of rice -well-milled, raw- is 356 kcal/100 g edible portion.[5)]) The purple rice is usually cooked with white rice, and the purple rice is 10% of the total rice cooked. Therefore, the experimental diet of the other group was supplemented with 4.9 g purple rice (Low content purple rice group (n = 5)). All of the diets contained the same levels of protein (20%), lipids (7%) and dietary fiber (5%). The rats were kept on this diet for 28 d. The diet and water were available at all times. The animals were deprived of their diet at 0900 on the 28th d, but allowed free access to water until killing, which was performed 4 h later. Blood was collected from the heart and left to clot at room temperature to obtain plasma. The liver was quickly removed, washed with cold 0.9% NaCl, blotted on filter paper, and weighed. The plasma and liver were stored at -80℃ until analyses were done. Aliquots of the liver were also preserved in methanol and stored at 4℃ until analyses of the lipid contents were performed.

Lipid analyses. The plasma total cholesterol, HDL-cholesterol, triglyceride, phospholipid, and NEFA concentrations were determined by an enzymatic method using a Cholesterol C-test, HDL-Cholesterol-test, Triglyceride G-test, Phospholipid C-test, and NEFA C-test, respectively. All test kits were obtained from Wako Pure Chemical Industries, Osaka, Japan. The difference between total cholesterol concentration and HDL-cholesterol concentration was regarded as (VLDL+LDL)-cholesterol concentration.

Total lipids from the liver were extracted according to the procedure described by Folch *et al.*[6)] After portions of the chloroform phase had been dried under nitrogen, the cholesterol,[7)] triglyceride,[8)] and phospholipid[9)] contents were determined.

The plasma thiobarbituric acid-reactive substance

Table 2. Initial body weight, food intake, body weight gain, and liver weight in rats fed purple rice.

Measurement	Control	Low content purple rice	High content purple rice
Initial body weight (g)	59.9 ± 1.1	59.9 ± 1.7	59.9 ± 1.6
Food intake (g/28d)	465.7 ± 15.1	483.9 ± 5.1	472.4 ± 8.1
Body weight gain (g/28d)	212.3 ± 7.4	226.4 ± 4.1	216.0 ± 4.2
Liver weight (g/100g body weight)	4.36 ± 0.15	4.19 ± 0.18	4.01 ± 0.17

Each value represents the mean ± SEM for six rats (Control group) or five rats (each, Low content purple rice group and High content purple rice group).

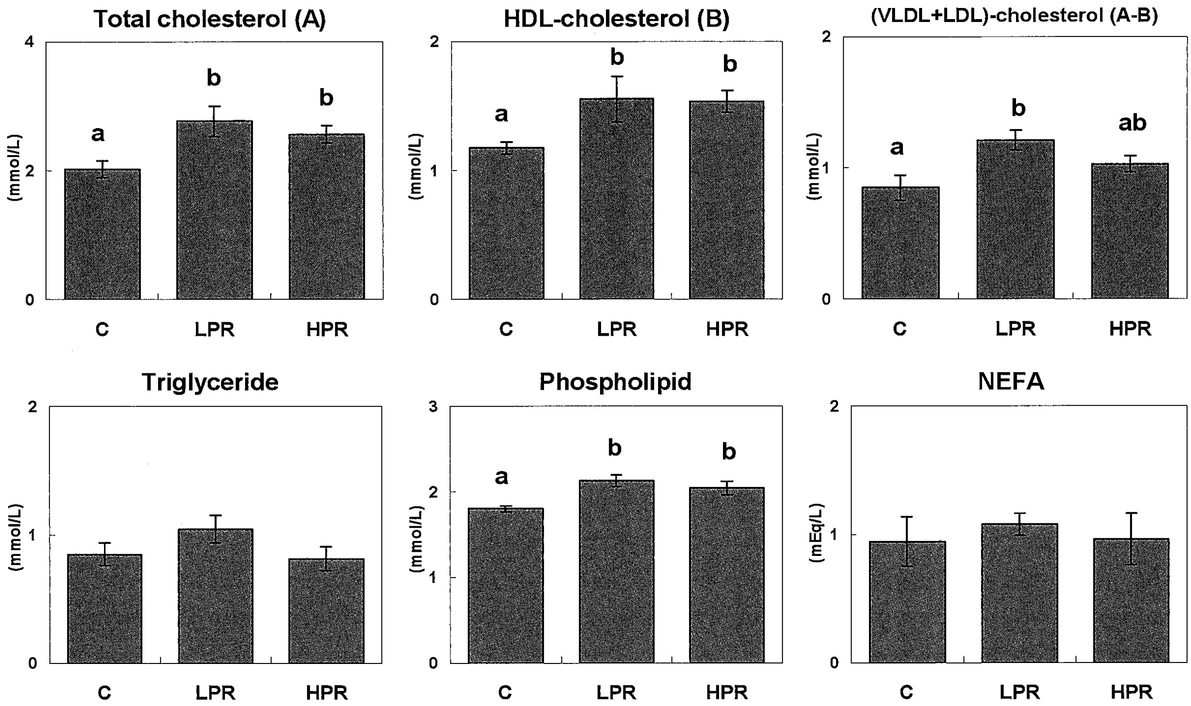

Fig. 1. Effects of dietary purple rice on plasma lipid concentrations in rats. Each value represents the mean for six rats (Control group) or five rats (each, LPR group and HPR group). Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test. C, basal diet (Control) group; LPR, low content purple rice diet group; HPR, high content purple rice diet group.

(TBARS) value was determined using a commercial kit purchased from Wako Pure Chemical Industries. The liver TBARS value was measured using the method described by Mihara *et al.*[10]

Statistical analyses. Results were expressed as mean ± standard error. Statistical analysis was carried out by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test. A significance level of $p < 0.05$ was used for all the comparisons.

RESULTS

Table 2 shows the initial body weight, food intake, and body weight gain for the duration of the 28 d of experimental feeding, and the relative liver weight at the end of experimental feeding. Food intake, body weight gain and liver weight were not significantly different among the three groups.

The plasma lipid concentrations are shown in Figure 1. The plasma total cholesterol concentration in the groups fed both low content purple rice and high content purple rice were significantly higher than that in the control group. In the lipoprotein cholesterol concentration, both high content purple rice and low content purple rice ingestion significantly enhanced the plasma HDL-cholesterol concentration. The plasma (VLDL+LDL)-cholesterol concentration in the group fed the low content purple rice was significantly higher than that in the control group. However, the high content purple rice feeding did not affect the plasma (VLDL+LDL)-cholesterol concentration. There were no significant differences in the plasma triglyceride and NEFA concentrations among the three groups. The plasma phospholipid concentration was significantly increased by both the high and low content purple rice ingestion.

The liver lipid contents are shown in Figure 2. There was no significant difference in the liver cholesterol content among the three groups. The liver triglyceride content in the groups fed the low content purple rice and high content one was not also significantly different from the control group, although the high content purple rice ingestion tended to decrease the liver triglyceride content. The liver phospholipid content in the group fed the high content purple rice was significantly higher than that in the control group. However, the low content purple rice ingestion did not affect the liver phospholipid content.

Figure 3 shows the TBARS values of the plasma and liver. The plasma TBARS value in the group fed the low content purple rice was not significantly different from the control group. However, the high content purple rice ingestion

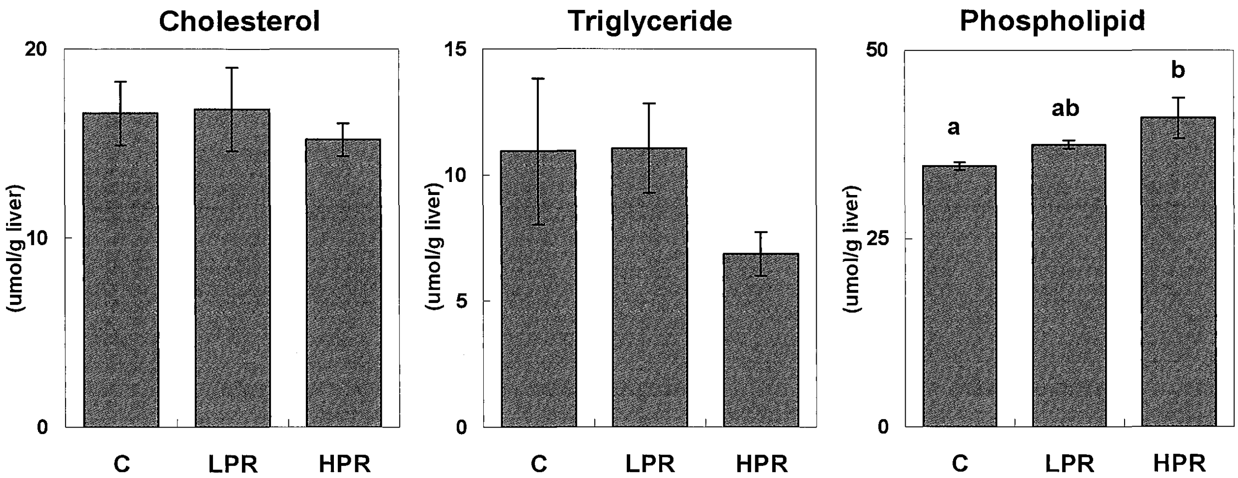

Fig. 2. Effects of dietary purple rice on liver lipid contents in rats. Each value represents the mean for six rats (Control group) or five rats (each, LPR group and HPR group). Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test. C, basal diet (Control) group; LPR, low content purple rice diet group; HPR, high content purple rice diet group.

significantly decreased the plasma TBARS value. The liver TBARS value was not changed by both the high and low content purple rice ingestion.

DISCUSSION

This study was performed to evaluate the effects of dietary purple rice on the plasma and liver lipid levels in rats. The purple rice ingestion significantly increased the plasma total cholesterol and HDL-cholesterol concentrations. The mechanism of the plasma HDL-cholesterol lifting action might be caused by the specificity of the purple rice protein. Dietary protein changes the cholesterol concentration in the serum or plasma. For example, dietary proso millet protein concentrate increased the plasma HDL-cholesterol concentration in comparison to the casein diet in mice.[2,3] Dietary fish protein ingestion increased the serum HDL-cholesterol concentration compared with the casein diet in rabbits.[11,12] There is a possibility that purple rice protein might also be related to the plasma HDL-cholesterol lifting action seen in the present study. Cholesterol is an animal sterol which plays many important

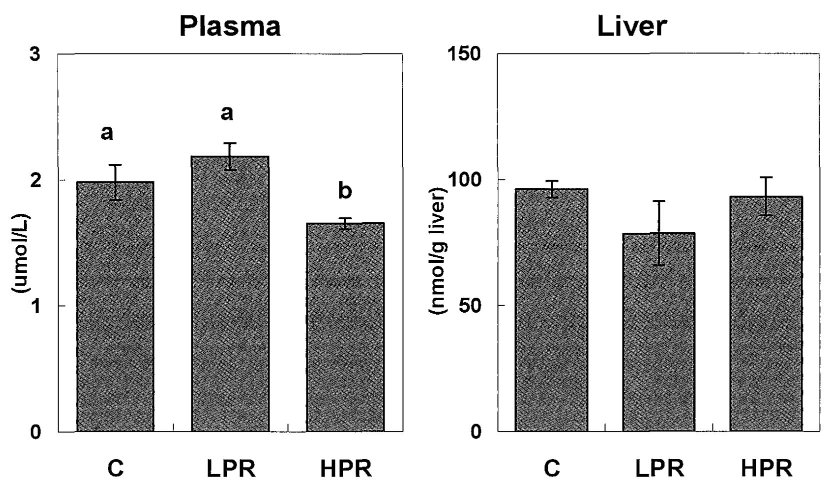

Fig. 3. Effects of dietary purple rice on plasma and liver thiobarbituric acid-reactive substance (TBARS) values in rats. Each value represents the mean for six rats (Control group) or five rats (each, LPR group and HPR group). Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test. C, basal diet (Control) group; LPR, low content purple rice diet group; HPR, high content purple rice diet group.

roles in the body. For example, cholesterol is a precursor of steroid hormones and bile acids. Cholesterol transport to extrahepatic tissues is primarily ensured by LDL while HDL retrieves cholesterol from extrahepatic tissues and carries it back to the liver in blood, and its concentration is inversely related to the risk of coronary heart disease. The result in the present study suggests that the dietary purple rice might affect the beneficial action of the cholesterol metabolism.

High content purple rice ingestion significantly decreased the plasma TBARS value. The TBARS value is an index of lipid peroxidation in blood or tissues, so the purple rice ingestion might have exerted an antioxidative effect in blood in the present study. Anthocyanin pigments are polyphenol and widely distributed in foods such as fruits and vegetables. These pigments have an antioxidative activity[13)] and play an important role as dietary antioxidants for the prevention of oxidative damage caused by active oxygen radicals. The ingestion of red wine, which is rich in anthocyanin, elevated the LDL antioxidative activity in human serum.[14)] Anthocyanin ingestion significantly decreased the serum TBARS value in rats.[15)] The pigments of purple rice are also anthocyanin. The pigment extracted from one kind of colored rice was anthocyanin and had an antioxidative property.[16)] The purple rice used in the present study also contains anthocyanin.[17)] Therefore, the reduction of the plasma TBARS value seen in the present study might be related to the antioxidative action of anthocyanin.

In conclusion, the purple rice may exert an antiatherosclerotic action along with the increase in the plasma HDL-cholesterol concentration, and an antioxidative action through the suppression of the plasma TBARS value. These findings suggest that the ingestion of purple rice is beneficial for the lipid metabolism.

REFERENCES

1) Kawasaki, M., Sakamoto, H., Takahashi, R., Nishita, Y., Kano, M., Kon, Y., and Sugawara, C., Effects of buckwheat (*Fagopyrum esculentum*) hot-water extracts on plasma and liver lipid levels in rats, *Bulletin of Morioka Junior College, Iwate Prefectural University*, **8**, 7-12 (2006).
2) Nishizawa, N., Oikawa, M., and Hareyama, S., Effect of dietary protein from proso millet on the plasma cholesterol metabolism in rats, *Agric. Biol. Chem.*, **54**, 229-230 (1990).
3) Nishizawa, N., and Fudamoto, Y., The elevation of plasma concentration of high-density lipoprotein cholesterol in mice fed with protein from proso millet, *Biosci. Biothchnol. Biochem.*, **59**, 333-335 (1995).
4) Reeves, P. G, Nielsen, F. H., and Fahey, G. C. Jr., AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet, *J. Nutr.*, **123**, 1939-1951 (1993).
5) Standard tables of food composition in Japan, Fifth revised edition, Resources Council, Science and Technology Agency, Japan (2000).
6) Folch, J., Lees, M., and Sloane-Stanley, G. H., A simple method for the isolation and purification of total lipides from animal tissues, *J. Biol. Chem.*, **226**, 497-509 (1957).
7) Zak, B., Simple rapid microtechnic for serum total cholesterol, *Am. J. Clin. Path.*, **27**, 583-588 (1957).
8) Van Handel, E., Suggested modifications of the micro determination of triglycerides, *Clin. Chem.*, **7**, 249-251 (1961).
9) Chen, P. S., Toribara, T. Y., and Warner, H., Microdetermination of phosphorus, *Anal. Chem.*, **28**, 1756-1758 (1956).
10) Mihara, M., and Uchiyama, M., Determination of malonaldehyde precursor in tissues by thiobarbituric acid test, *Anal. Biochem.*, **86**, 271-278 (1978).
11) Bergeron, N., and Jacques, H., Influence of fish protein as compared to casein and soy protein on serum and liver lipids, and serum lipoprotein cholesterol levels in the rabbit, *Atherosclerosis*, **78**, 113-121 (1989).
12) Bergeron, N., Deshaies, Y., and Jacques, H., Dietary fish protein modulates high density lipoprotein cholesterol and lipoprotein lipase activity in rabbits, *J. Nutr.*, **122**, 1731-1737 (1992).
13) Tsuda, T., Ohshima, K., Kawakishi, S., and Osawa, T., Oxidation products of cyanidin 3-*O*-β-D-glucoside with a free radical initiator, *Lipids*, **31**, 1259-1263 (1996).
14) Kondo, K., Matsumoto, A., Kurata, H., Tanahashi, H., Koda, H., Amachi, T., and Itakura, H., Inhibition of oxidation of low-density lipoprotein with red wine, *Lancet*, **344**, 1152 (1994).
15) Tsuda, T., Horio, F., and Osawa, T., Dietary cyanidin 3-*O*-β-D-glucoside increases *ex vivo* oxidation resistance of serum in rats, *Lipids*, **33**, 583-588 (1998).
16) Isobe, Y., Morioka, M., Terahara, N., Komiya, T., and Narita, M., Antioxidative properties of the pigment from colored rice (Akamajiri-kuromai), *J. Cook. Sci. Jpn*, **39**, 247-250 (2006).
17) Itani, T., Tatemoto, H., Okamoto, M., Fujii, K., and Muto, N., A comparative study on antioxidative activity and polyphenol content of colored kernel rice, *J. Jpn. Soc. Food Sci. Technol.*, **49**, 540-543 (2002).

和文要旨　　ラットにおける血漿および肝臓脂質レベルに対する食餌紫黒米（朝紫）摂取の作用を検討した。ラットには紫黒米をヒトの米エネルギー摂取量に基づいて与えた。血漿総コレステロー濃度が高配合および低配合紫黒米摂取群の両者で対照群に比べて有意に上昇した。同時に，高密度リポたんぱく質 (HDL) コレステロール濃度が高配合および低配合紫黒米摂取群の両者で対照群に比べて有意に上昇した。血漿チオバルビツール酸反応物質 (TBARS) 濃度が，低配合紫黒米の摂取では有意差はみられなかったものの，高配合紫黒米の摂取により対照群に比べて有意に低下した。これらの結果より，紫黒米は血漿 HDL コレステロール濃度を上昇させることにより抗動脈硬化的に作用し，血漿 TBARS 濃度を抑制することにより，抗酸化的に作用している可能性が示唆された。